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May 19, 1961

Dr. Paul Berg
Department of Biochemistry
Stanford University
Palo Alto, California

Dear Paul:

As far as I know the only system available at present for separating the 2' and 3' isomers of pseudo-U is ion-exchange chromatography, using systems similar to the one I used in my paper (Biochem. Biophys. Res. Comm. 3, 504 (1960)). The 2' isomer that I originally sent you was isolated from commercial yeast RNA by this method. The fractions containing pseudo-U were pooled and then rechromatographed. The 2' isomer I sent you was isolated from the valley between the 2'-3' isomers. As you can see from the figure in the paper it is very doubtful that there is contamination with uridine 2',3' phosphates. There are two better possibilities for contamination. The most likely candidate is ribothymidylic acid. Since the enolic pK's of pseudouridylic and thymidylic acids are = 9.43 and 10 respectively, pseudouridylic acid should have a greater electrophoretic mobility at a pH of around 9. In your letter you did not give the conditions for your electrophoresis or the mobility of the "uridylate?" contaminant relative to the 2' isomer. I would be interested in knowing these conditions. Also, could you give me an estimate as to the amount of this contaminant? Because of its methyl group, ribothymidylate would be expected to have a greater mobility than pseudouridylic acid in a paper chromatographic system. From personal experience this would hold for the isopropanol-HCl and isobutyric-NH_3 systems. Again, what systems have you used and what is the relative mobility of the 2' isomer to its contaminant? I have found that the 3' isomer I sent you is chromatographically pure in isobutyric-NH_3.

Another possible contaminant of pseudouridylic acid is UMP-5'. According to some workers, UMP-5' has been found in alkaline digests of RNA - the fact that the extent of this contamination is not reproducible would indicate that it is probably due to incomplete extraction of the acid solubles. If this is the contaminant, then the UMP-5' will have greater electrophoretic mobility in borate buffer, and a greater Rf in isopropanol-HCl or isobutyric-NH_3 solvents. Periodate consumption or treatment with a 5' phosphatase would be further confirmation if there is 5' UMP, besides, of course, typical u.v. curves.

In regards to your contribution to Methods in Enzymology - Fred Bergmann had given me the impression that you were writing an article that dealt with the isolation of a.a. acceptor RNA. I have a copy of your procedure using sodium lauryl sulfate, norite, and DEAE. I was wondering if you had made any recent modifications in this procedure. What kind of yields of a.a. acceptor RNA do you get (mg RNA/wet wt. cells)? Have you tried other methods - such as phenol extraction - to prepare acceptor RNA?

Sincerely yours,

Ted

T. R. Breitman
Biochemical Research Section

TRB:mjh